RARAF

THE RADIOLOGICAL RESEARCH ACCELERATOR FACILITY

An NIH-Supported Resource Center

WWW.RARAF.ORG

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Research Using RARAF

Both the Microbeam and the Track Segment Facilities continue to be utilized in various investigations of the response to radiation exposure. This year the number of biological experiments has declined somewhat, with newer technological developments on the Super Microbeam displacing machine availability for users. The track segment facility and our neutron capabilities remain in operation providing users averaged particle irradiation for population studies. We also have the new development of a "FLASH" irradiation system delivering high doses in fractions of a second.

Experiments

Listed in Table I are the experiments performed using the RARAF Singletron between January 1 and December 31, 2017 and the number of shifts each was run during this period. Half shifts are assigned when experimental time is shared among several users (e.g., track segment experiments) or when experiments run for significantly more or less than an 8-hour shift. Use of the accelerator for experiments was 47% of the regularly scheduled time (40 hours per week). Nine different user experiments were run during this period. Three experiments were undertaken by members of the CRR, supported by grants from the National Institutes of Health (NIH), specifically the National Cancer Institute (NCI), the National Institute of Allergies and Infectious Diseases (NIAID) and the National Institute of Biomedical Imaging and Bioengineering (NIBIB). Six experiments were performed by external users, supported by grants and awards from the Department of Energy (DoE), the Department of Defense (DoD), the NIH, the National Aeronautics and Space Administration (NASA), the National Science Foundation (NSF), the National Cancer Institute (NCI), and internal funding from Cornell

Table I. *Experiments Run at RARAF January 1 - December 31, 2017*

Exp No.	Experimenter	Institution	Exp. Type	Title of Experiment	Shifts Run
113	Alexandra Miller	AFRRI	Biol.	Role of alpha particle radiation in depleted uranium-induced cellular effects	3.5
162	Lubomir Smilenov	CRR	Biol.	Mouse irradiation using IND spectrum neutrons	4
165	Helen Turner	CRR	Biol.	Mouse/blood irradiation using IND spectrum neutrons	2
172	Susan Bailey	Colorado State University	Biol.	Targeted telomeric damage and the persistent DNA damage response	0.5
173	Ekaterina Dadachova	Albert Einstein College of Medicine	Biol.	Comparison of fungal cell susceptibility to external alpha particle beam radiation versus alpha particles delivered by ^{213}Bi-labeled antibody	4
174	Gordana Vunjak-Novakovic	Columbia University	Biol.	Micro proton induced x-ray emission of bone/cartilege grown on artificial scaffolds	0.5
175	Constantinos Broustas/ Sanjay Mukherjee	CRR	Biol.	Mouse/blood irradiation using IND spectrum neutrons	2
178	Alejandro Carabe-Fernandez	University of Pennsylvania	Phys.	Microdosimetric and radiobiological characterization of new Si-based microdosimeters using particle microbeams	4
179	John Ng	Cornell University	Biol.	Effect of LET on immunotoxicity	26.5
180	Francesco d'Errico	Yale University	Phys.	Neutron bubble spectrometery	5
181	Joel Greenberger	Univ. of Pittsburgh	Biol.	Mouse irradiation using IND spectrum neutrons for radiation mitigator effectiveness studies	3

University. One of these experiments was a collaboration between RARAF/CRR staff and an outside user. Brief descriptions of these experiments follow.

Dr. Alexandra Miller of the Armed Forces Radiobiological Research Institute (AFRRI) continued studies using the Track Segment Facility to evaluate depleted uranium (DU) radiation-induced carcinogenesis and other late effects employing *in vitro* models and to test safe and efficacious medical countermeasures (Exp. 113). One objective of this study has been to determine if phenylbutyrate (PB), a histone deacetylase inhibitor and epigenetic effector, can mitigate neoplastic cell transformation induced by different qualities of radiation, and if so, to identify which adverse epigenetic mechanisms are involved and potentially reversed by PB. This also would be of interest for Space missions and alpha particle exposures from accidental releases. Track segment irradiations with ^{4}He ions were performed on human small airway epithelial cells (SAECs) and growth rate, transformation, and genomic instability were quantified. Irradiation of SAECs overcame contact inhibition and caused an increase in transformation frequency and induction of gene amplification, i.e., genomic instability. Treatment with PB following irradiation resulted in a significant suppression of transformation frequency and gene amplification. Studies are ongoing to evaluate the impact of PB treatment on changes in DNA methylation caused by irradiation with ^{4}He ions.

Dr. Miller also instituted a study using her SAEC line in a comparison study of the Columbia IND-spectrum Neutron Facility (CINF) at RARAF and the reactor neutron spectrum irradiator at AFRRI. This intercomparison work is being supported by AFRRI for their systems analysis by comparison to other facilities. Results of this work will be shared with RARAF and the broader community to further understanding of the effects of differing neutron energy spectra.

Drs. Helen Turner, Constantinos Broustas, and Sanjay Mukherjee made use of CINF to study the effects of the spectrum irradiation of human blood samples and mice. This work is supported by the U19 Columbia CMCR for the development of biodosimetry tools for a radiologic event. Mice were irradiated with up to 2 Gy of neutrons and comparison mice were given up to 4 Gy of x-rays using the Westinghouse orthovoltage x-ray system. Blood samples were given up to 2 Gy of neutron spectrum dose and 4 Gy of x-rays. There were also mice given up to 1 Gy of neutrons and then a secondary dose of x-rays to simulate a mixed field. The mice were sacrificed and blood was collected and subjected to whole genome gene expression analysis. Blood samples were also scored for micronucleus and γH2AX foci to determine dose response. Some animals were also held in metabolic cages for collection of urine and feces, for metabolomics analysis.

Dr. Susan Bailey from Colorado State University works on telomere length and damage effects on the health and viability of cells. She uses the RARAF microbeam to target and irradiate telomeres in cells. The work performed this year focused on telomere degradation following targeted nuclear irradiation. The experiment was used also by the RARAF staff as a baseline imaging test for the imaging of telomeres, using labels of interest to Dr. Bailey, with the new super resolution microscope as that facility will become available early next year.

Dr. Ekaterina Dadachova at the Albert Einstein College of Medicine, working with Igor Shuryak of the CRR, has been developing radioimmunotherapy (RIT) for treatment of *Cryptococcus neoformans* infections using ^{213}Bi-labeled antibodies specific to the cryptococcal capsule. She is performing a comparison of fungal cell susceptibility to external α-particle beam radiation versus α particles delivered by the bismuth-labeled antibodies (Exp. 173). Fungi grown to stationary phase in defined minimal medium were suspended in solution. As for other experiments, the solution was formed into a thin layer with a known uniform thickness under a cover slip. The fungi were irradiated with doses of 1 to 80 Gy of 125 keV/μm ^{4}He ions. Results so far indicate that: a) *C. neoformans* is more sensitive to external beam α particles than to external γ rays; b) α particles delivered by the capsule-binding antibodies may be more cytocidal to the *C. neoformans* cells than external beam α particles. This work has expanded in the past year to include proteomic, transcriptomic and metabolomic research into the radioresistance observed in these fungi.

Dr. Gordana Vunjak-Novakovic uses our charged particle microbeam facilities for PIXE analysis of cartilage-bone interfaces looking at chemical composition of the two materials as they interface and progress through the life cycle. The change in calcium concentration in both materials through the development of arthritis is of high interest in arthritis care and prevention. This past year, the neutron microbeam line has been modified to allow higher beam currents on target for more rapid data acquisition, enabling the performance of this work at the neutron microbeam endstation. Samples from both sacrificed animals and laboratory constructs on artificial scaffolds are being measured. The design of the artificial scaffolds could lead to the ability to grow bone and cartilage replacements in the laboratory from a patient's own stem cells for joint reconstruction and repair.

Dr. Carabe-Fernandez of the University of Pennsylvania is developing silicon 3D radiation microsensor arrays, capable of quantifying deposited energies within micron-sized targets. Compared to traditional tissue equivalent proportional counters, these detectors do not require a gas supply, operate at low voltages, are light and easily portable, and have a fast response. The goal of this project is to use the targeting

ability of the microbeam to characterize individual microsensors within the microdosimeter array. Different microdosimeters of different dimensions (diameter, depth and pitch) representing different cell types, will be exposed and the derived relative biological effectiveness (RBE) from mechanistic biophysical models (e.g. MKM and LEM) will be calculated. The experimental RBE obtained from clonogenic assays of individual cells exposed to the microbeam will also be obtained and compared to that obtained from the microsensors. This will allow: 1) characterization of the microdosimetric properties of each individual microsensor as well as study crosstalk between the sensors in an array; 2) validation of the microsensors as viable instruments to calculate RBE; 3) determination of new features required to develop current microsensor technology to a new generation that allows more precise RBE measurements.

Dr. John Ng has expanded his work significantly with the help of the RARAF staff. Building on his significant experience in clinical cancer treatment, he is heading a project looking for immune response signals from cells after irradiation using particles of different LET. The hope is to determine effects of targeted radiotherapy with specifically chosen particle energies that can be combined with immunotherapy to increase the efficacy of both for the treatment of many types of cancers. This study has focused on a mammary tumor cell line that was developed at Cornell University for the study of the immune response. The study monitors the relocation of calreticulin from the ER to the cell membrane and the release of HMGB-1 and ATP into the intercellular matrix/media. These three responses are indicative of immunogenic cell death – a radiation-induced response that activates the immune system. The experiment makes use of the RARAF track segment irradiator as a source for particles of different LETs (from 10 to 160 keV/μm). Studies this year explored the higher end of the LET range (65-160 keV/μm). The results are promising in that they show a peaked response in all three assays at ~110 keV/μm. We are in the process of confirming these results, and we look forward to further exploring low LETs (10, 25 and 40 keV/μm), and expanding these studies to other cancer as well as normal tissue cell lines.

Dr. Francesco d'Errico from the Dept. of Physics at Yale University has developed a technique for neutron spectroscopy based on measurements of bubble formation in superheated emulsions. Dr. d'Errico performed extensive studies of the response of these detectors as a function of emulsion temperature and neutron energy. The preliminary data obtained was used in several applications for further funding and Dr. d'Errico expects to return in 2018.

Dr. Joel Greenberger from the University of Pittsburgh has initiated mouse irradiation studies with our IND spectrum irradiator to test radiation injury mitigator drugs he has developed through their U19 CMCR project. This is a long-term project in collaboration with the Columbia CMCR, which will run through the next year looking at $LD_{50/30}$ changes with respect to drug application.

Development of Facilities

Development continued on a number of our irradiation facilities and capabilities for imaging and irradiating biological specimens:

- Focused particle microbeams
- CINF
- FLASH irradiation platform
- Advanced imaging systems
- Targeting and manipulation of cells
- New cell analysis tools
- Small animal systems

Focused particle microbeams

The Super Microbeam Phase 1 construction was completed last year. The current beam size is 3.5 μm with further alignment optimization underway. While not the ultimate beam size for the Phase 1 (250 nm), this beam spot size has allowed the recommencement of microbeam operations at the Super Microbeam endstation. The ultimate size of the beam for Phase 1 will be 250 nm, which we hope to achieve early in 2018. Phase 2 will be undertaken in the fall of 2018 with the reinstallation of the electrostatic double lens as the first focusing element.

During the redevelopment of our electrostatic/Super Microbeam system, the permanent magnet microbeam (PMM) was used as our primary charged particle microbeam. This system is also the microbeam endstation for the development of our Flow and Shoot (FAST) microfluidics irradiation system, the capillary electrophoresis (CE) system, and the automated cell picking system. The PMM has all of the irradiation capabilities of the electrostatic microbeam except the sub-micron beam spot size. The PMM also does not have the electrical breakdown potential from failures of the vacuum window making it an ideal initial testbed for all our new technologies.

Columbia IND-Spectrum Neutron Facility (CINF)

CINF was completed in 2014 and has been extensively used since. This year saw the irradiation of mice, fresh whole blood samples, and plated cell lines

The fast neutron irradiation source was designed to generate the neutron spectrum observed from the "Little Boy" atomic bomb at Hiroshima at 1.5 km from ground zero. This field is generated through the reactions $^{9}Be(d,n)^{10}B$ and $^{9}Be(p,n)^{9}B$ using a mixed beam of monoatomic, diatomic and triatomic protons and deuterons. The RARAF Singletron uses a gas mixture of hydrogen to deuterium of 1:2, which feeds into the RF plasma ion source. This irradiator is on the 0º beam line, as any bending of the beam to get to a target would separate the 6 different beams and prevent spectrum generation.

The neutron spectrum was verified using two proton recoil detection systems, 2"-diameter 2"-thick liquid scintillator for energies >1 Mev and a 1.5"-diameter spherical gas proportional counter with 3 atmospheres of hydrogen gas for <1 MeV. Using MCNPX-PoliMi Monte Carlo simulations to calculate the exact response functions of the detectors, it is possible to reconstruct the spectrum from the readout of the detectors in the neutron field.

The base irradiation dose rate has been calibrated to deliver a dose rate of up to 2 Gy/h (with an additional gamma-ray contribution of 18%).

FLASH irradiation platform

The FLASH irradiation platform is designed for ultra-high dose rate particle irradiations. The proton beam from the accelerator is rapidly switched on and off by sweeping it across the beam window aperture. By precise control of the beam current and timing of the beam sweep, we have tested dose rates from 2 Gy/min up to more than 1000 Gy/sec, delivering a full range of therapeutically relevant doses to samples confirmed through film dosimetry measurements.

This year we verified the dosimetry, cellular survival curves demonstrating delivered dose effects, and conducted preliminary work for tissue models and live animal (mouse) models.

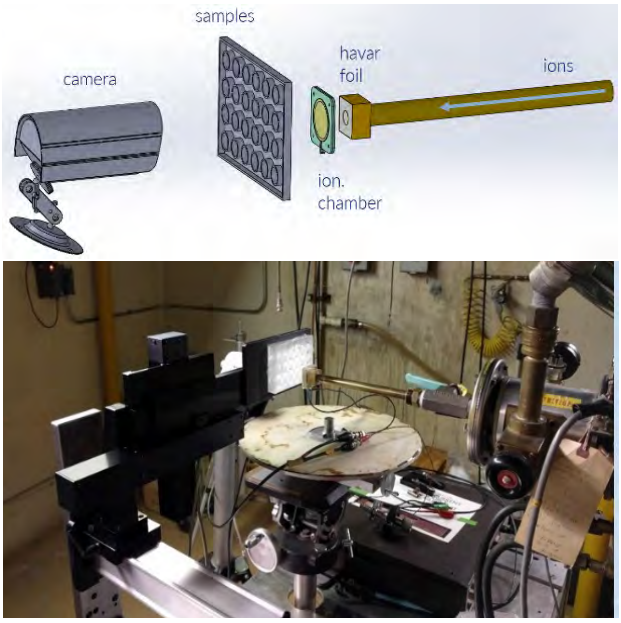

Figure 1. *Schematic layout of the FLASH irradiator (top) and photo of the end station on the "cave" beamline with the FLASH setup (bottom).*

Advanced imaging systems

We continue to develop new techniques to obtain two- and three-dimensional images of cells, reduce UV exposure and improve resolution.

Real-time imaging

Short-term biological effects that happen within seconds to the first few minutes after irradiation set the stage for later effects. Real-time imaging and observation of the short-term effects will give experimenters insight into their endpoints. Techniques have been developed using our EMCCD camera and our fast switching SOLA LED light source to acquire images with several frames per second to observe the short-term effects of irradiation on a timescale of minutes to hours following irradiation.

Multi-photon microscope with the UV microspot

The multi-photon microscope was developed several years ago and integrated with the charged particle microbeam irradiator. This microscope, through the long wavelength incident laser, allows in depth imaging of 3D tissues and small animals, such as *C. elegans* and zebrafish embryos. This is achieved using the sectioning capability of the multi-photon effect where the photon density increases to generate constructive interference producing a 3D voxel of half the wavelength, twice the energy photons that can locally excite fluorophores and/or other fluorescent effects (e.g. auto fluorescence and second-harmonic generation). This 3D voxel is then scanned through a single layer and stepped through the sample using the nanoprecision z-stage, generating a stack of 3D slices of the sample that are reconstructed into 3D images.

If the intensity of the laser is increased, at the area of constructive interference, there can be a 3-photon interference resulting in a voxel with 1/3 of the wavelength (three times the energy) typically generating a voxel of UV light—the UV microspot. The UV microspot can be used to induce damage within a 3D target.

STED

We are developing a Stimulated Emission Depletion (STED) super resolution microscope system with optical resolution of 75 nm in combination with our super microbeam to achieve compatible imaging resolution and beam spot size. The STED system at RARAF builds on the multi-photon microscope, using it as the primary excitation laser. A second continuous wave laser is added in parallel with the multi-photon laser. Using polarization optics, the second laser projects a donut shaped point spread function around the excitation spot of the multi-photon system. With proper selection of the second laser wavelength and with sufficient intensity, the second laser will deplete the fluorescent states around the excitation spot, allowing fluorescence from the center of the donut, which will be reduced to nanometer sizes.

The STED development continues on the microbeam endstation. We are upgrading the microbeam endstation with new custom control software in preparation for the heavy ion microbeam upgrade. This upgrade includes new control software for the multi-photon microscope with integrated fast timing for the gSTED development.

This work is ongoing and we expect gSTED imaging coming soon in 2018.

Targeting and manipulation of cells

We have the capability to fabricate microfluidic devices in hard plastics, such as acrylic, and soft plastics, such as polydimethylsiloxane (PDMS). The micro-milling machine installed at RARAF has software to produce parts designed using the Solid Works computer-aided design (CAD) program. This system has been used to manufacture the single-cell dispenser and the microfluidics chips for the cell sorter and microFACS systems (described below). Several new microfluidic systems are being developed to target, manipulate and analyze cells.

Cell picker

Picking individual cells that are adhered to a microbeam irradiation dish is one of the methods to isolate and subsequently load a cell into one of the microfluidic analysis chips being developed in the P41 grant. Previously, this capability was incorporated into the Permanent Magnet Microbeam endstation as a semi-automated device, which is part of the microbeam control software and includes joystick control of robotic movements. In the past year, we have worked to improve the workflow of the cell picker. We optimized the picking conditions, including the amount of liquid on the cells, staining method, and imaging setup, as well as the general technique of locating a cell, dispensing trypsin and then aspirating a single cell. We are currently working on improving our picking speed and the efficiency of picking a single cell.

Cell dispenser

Development of the single cell dispenser has continued with a focus on improving electrical signal quality and testing a complete system with cells. We have improved the electrical signal quality, which is used to detect when a cell is passing over the microelectrodes within the device, by making the connection to the electrode more mechanically robust. This robust connection reduces the noise and makes triggering off of a cell detection event easier. Testing of the dispenser system has moved from using beads to using cells in suspension. To extend the period we could test cells without them losing shape (due to their death since they are out of an incubator), we began work with chemically fixed suspended cells. We also applied a crystal violet dye to the fixed cells to enable us to view them easily both within the microfluidic device and within a dispensed droplet. We continue testing the complete system and are evaluating the ability of the system to eject a single cell autonomously.

MicroFACS

We have continued development of the microfluidic Fluorescence-Activated Cells Sorting (microFACS) system to combine flow cytometry and sorting with our other microfluidic irradiation and dispensing technologies.

The microFACS system uses Dean vortex drift flow focusing to entrain the samples into a sheath flow focused column for flow cytometry detection in the main channel. The sample is illuminated with a laser through fiber optic coupling with the fluorescent output also detected through fiber optic coupling. The combination of fiber optics and microfluidics will allow the microFACS to be coupled to the other microfluidic systems, in close proximity to the microbeam endstations.

AMOEBA

The Automated Microbeam Observation Environment for Biological Analysis (AMOEBA) system allows for long term experiments where cells can be exposed using the microbeam and continuously observed for over 36 hours. The system, which typically monitors and controls temperature, pH, and humidity on a microbeam endstation, is a modular configuration that can be adjusted for any number of experimental conditions. The system is run through custom software that can monitor multiple inputs simultaneously and make appropriate changes to control the environment. Investigators who wish to use the AMOEBA for their microbeam experiments can work with the RARAF team to configure the AMOEBA for their needs.

The microAMOEBA is similar to the AMOEBA, which has been designed to work around the existing microbeam irradiation protocol, because it also has the goal of carefully controlling the environment during a microbeam experiment. The microAMOEBA is unique because it aims to specifically control the microenvironment around cells with the added goal of enabling faster changes of controlled parameters than would be possible with the AMOEBA, because of the significantly reduced control volume. The microAMOEBA is designed to operate using the same control software and modules as the AMOEBA system, while the sensors and actuators for the system are made within a microfluidic system. We construct the microAMOEBA using a silicon substrate, which can contain all necessary electrical connections and a thin window to allow the microbeam to reach the cells, and an attached microfluidic structure made of PDMS. The PDMS not only acts as the cell culture chamber but it also allows for control of the dissolved oxygen through controlled diffusive transport.

New cell analysis tools

CE-LIF

We have finished construction and begun testing of our Capillary Electrophoresis – Laser Induced Fluorescence (CE-LIF) system to provide our users with the capability of measuring reactive oxygen species within individual cells immediately after irradiation. The nanoliter input volumes makes this system ideal for single-cell, small-scale biochemical analysis.

The CE-LIF system at RARAF: The grounded end of a 50 μm bore capillary is brought to the cell using the semi-automated cell picker. Once a cell is aspirated into the capillary, 20-30 kV is applied between the grounded end of the capillary and the Laser Induced Fluorescence (LIF) system, enclosed in a light tight insulating box. This results in two superimposed flow modalities experienced by the analytes: (1) Electrophoretic flow, responsible for separating the analytes by charge and Stokes radius; (2) Electroosmotic flow, which drives the buffer and analytes (regardless of polarity) toward the detector. The electroosmotic flow is much stronger than the electrophoretic flow, ensuring that all analytes will reach the detector. In the LIF system, the analytes are hydrodynamically focused into the path of a laser, with the light collected perpendicularly and detected by a high-sensitivity spectrometer. We have recently acquired a deep cooled Bayspec spectrometer, providing highly sensitive detection of fluorescent molecules.

Small animal systems

Mouse Ear Irradiation

Investigations of radiation-induced bystander effects have been conducted in cell cultures and 3-D systems *in vitro*. The next logical step was to develop and implement microbeam irradiation protocols to study effects in living organisms. We have developed a mouse ear model for *in vivo* bystander studies. With an average thickness of 250-300 μm, this model can be used to investigate radiation-induced bystander effects with a 3-MeV proton microbeam having a range of 134 μm.

Using gentle suction, the ear of an anesthetized mouse is flattened onto the underside of a flat plate of a custom-made holder. The flattened mouse ear is then placed over the microbeam port and cells along a line of the ear are irradiated with the proton microbeam. At chosen times after irradiation, mice are sacrificed and a punch of the ear is collected. Tissues are then fixed, paraffin-embedded and cut in 5-μm sections perpendicularly to the direction of the line of irradiation. The sections are then analyzed for biological endpoints (i.e., formation of repair protein foci, apoptosis) as a function of the distance from the irradiated line.

Mouse Phantom

The anatomically accurate mouse phantoms continue to be used in various capacities around the CRR. A crucial development in the past year has been comparison of the mouse phantom performance in a sample irradiation with a computer model of the same radiation. Two common irradiation protocols performed in the CRR, both using the Small Animal Radiation Research Platform (SARRP), were used to compare modeling with the physical phantom: a lung irradiation using the 3 mm square collimator and an abdominal irradiation using a 5 mm square collimator. The physical models were tested using radiochromic film strategically placed within the phantoms. The computer simulation was performed in MCNP and included the phantom as well as the SARRP. A comparison of the resulting radiation dose map, specifically in regions of very low dose outside of the target region, showed very good agreement between the physical models and the simulation. These results confirmed that this unique phantom is a good tool to accurately assess dose distribution.

The mouse phantoms were also used to help assess neutron dosimetry for projects in our Center for Medical Countermeasures against Radiation (CMCR). The phantoms were loaded into the exact positions used to irradiate mice in this experiment, thus allowing us to confirm that a uniform dose was received through the body of each mouse while it was rotated around the neutron source.

Singletron Utilization and Operation

Table II summarizes accelerator usage for the past year. The nominal Singletron availability is one 8-hour shift per weekday (~248 days per year); however, the accelerator is frequently run well into the evening, often on weekends, and occasionally 24 hours a day for experiments or development. Accelerator usage for online experiments and development was 51% of nominal day shifts. The off-line development, which did not require the accelerator while occupying the facility operations, used 83% of the nominal facility time. Combined with our maintenance and safety system operations, 151% of effective day shifts were utilized in 2017.

Accelerator use for radiobiology and associated dosimetry was about 85% that for last year and slightly below the average of the last 5 years. About 76% of the use for all experiments was for track segment irradiations, 5% for charged particle microbeam irradiations, and 19% for neutron irradiations. Approximately 84% of the experiment time was for studies proposed by external users, and 16% was for internal users.

On-line facility development and testing was about 24.5% of the available time, primarily for development and testing of the Super Microbeam solenoid focusing system, development of the FLASH irradiation platform, and dosimetry calibration amongst our different

Table II. *Accelerator Use, January 1 - December 31, 2017*
Normally Scheduled Shifts

Radiobiology and associated dosimetry	23%
Radiological physics and chemistry	3.5%
On-line facility development and testing	24.5%
Safety system	4.5%
Accelerator-related repairs/maintenance	10%
Other repairs and maintenance	2.5%
Off-line facility development	83%

irradiation platforms through film irradiations. There was also significant time dedicated to the multiple microfluidic and analysis tools using the PMM endstation. This is about average for the last five years and slightly more than last year.

The accelerator was opened twice in 2017, primarily for ion source replacements. The opportunity was also taken for general accelerator maintenance of diode replacement in the charging system, verification of the electrical generation systems, and measurements for the DREEBIT Heavy Ion Source installation. These openings typically take 5 days but were extended this year as needed for the other projects.

DREEBIT Heavy Ion Source

The DREEBIT Heavy Ion Source development is ongoing. The DREEBIT has been extensively tested this year using an off-line test bed. We reconfirmed the factory tests and made slight improvements that will facilitate the installation in the Singletron, in particular, the ultra-high vacuum needs of the EBIT system. We anticipate the mechanical installation testing of the DREEBIT in the summer of 2018, with the complete installation and operations in the Fall and Winter of 2018.

Training

REU

Since 2004, we have participated in the Research Experiences for Undergraduates (REU) project in collaboration with the Columbia University Physics Department. This is a very selective program that attracts highly talented participants. For 9-10 weeks during the summer, each student attends lectures by members of different research groups at Nevis Laboratories, works on a research project, and presents oral and written reports on his or her progress at the end of the program. Among other activities, the students receive a seminar about and take a tour of RARAF.

The 2017 REU participant at RARAF was Sabrina Campelo from Elon University. Sabrina worked with Andrew Harken on the development of the gSTED super resolution imaging system. The 10-week program involved the initial testing of the high-speed detection and measurement system for the gSTED. This included the reprogramming of the time-correlated single photon counting (TCSPC) card to work with our photomultiplier outputs and interfacing with our multiphoton microscopy system – the excitation beam for our STED system. Sabrina was instrumental in the development of the TCSPC system for the gSTED. She presented her work at the REU seminar at the end of the summer and wrote a report about it.

Group Training

In addition to training individuals at RARAF, staff members also participate in training courses presented at other facilities as a means of introducing microbeam concepts and experiments to a broader audience. This year, Andrew Harken lectured on "High/low LET microbeams" at the NASA Space Radiation Summer School, Brookhaven National Laboratory, Upton, NY, on June 20, 2017. Manuela Buonanno was the chair of the Experimental Methods Section for the NASA Summer School coordinating all the experiments for students.

Microbeam Training Course

The sixth RARAF microbeam training course "Single-Cell Microbeams: Theory and Practice" was given May 22-26, 2017. There were eight students participating, listed in Table III. Dr. Marcelo Vazquez returned as the director of the Microbeam Training Course.

The Course was adjusted to a 5-day schedule this year. The expansion from 3 days lead to a more relaxed atmosphere, more time for the students and instructors to interact, and the students had more time to work in the lab and on their beam-time proposals. The course generally followed the same pattern of technical lectures on Day 1, experimental lab sections on Day 2, further lectures on expanded topics on Day 3 with biology experiment tracking, final biology analysis and beam time proposal work on Day 4, and Day 5 was the final proposal reports and discussions. The expansion to 5 days was given a positive review by students and faculty.

A main feature of the course is the experimental design done by each of the students as if they were proposing to come to RARAF to do an experiment. The students work with the RARAF staff to devise potential experiments and then present these proposals at the end of Day 5 as a final demonstration of what they have learned from the course about the nature of microbeams and their potential applications.

Dissemination

Web site

The RARAF website design that was created in 2013 provides clear and effective presentation while improving access to content. Functional menus (including a home page rotating-picture menu) were designed to make navigation through the content easy and interesting, with a hierarchical structure from general information, suitable for a general or non-science audience, to more-detailed technical content.

The site contains information on microbeams in general, as well as detailed technical information on our various microbeams; *in-vitro* and *in-vivo* endpoints that we use; details of available on-line and off-line imaging capabilities; microfluidic systems we are developing; other charged particle and neutron irradiation facilities available at RARAF; our on-line training course materials; publications lists; information on RARAF contacts and directions to the facility. The site is periodically updated to include new radiation facilities, cell handling and analysis capabilities, publications and other information.

Table III. *Students for the sixth RARAF Microbeam Training Course.*

Name	*Position*	*Affiliation*
Sofia Barbieri	Ph.D. Student	University of Pavia, Italy
Pavel Blaha	Ph.D. Student	Czech Technical Universtiy, Czech Republic
Brian Canter	Ph.D. Student	Rutgers University
Kadeshia Earl	M.D./Ph.D. Student	Texas Southern University
Sunny Narayanan	Ph.D. Student	Texas A&M University, Health Science Center
Anna Michaelidesova	Ph.D. Student	Nuclear Physics Institute, ASCR, Czech Republic
Federico Picollo	Professor of Physics	University of Turin, Italy
Emiliano Pozzi	Researcher	CNEA, Argentina

Virtual training course

We have developed an on-line virtual microbeam training course, based on the three-day microbeam training courses. This on-line course was designed to give interested physicists and biologists who could not attend in person a thorough introduction to microbeam technology.

The goal of the online course, as for the face-to-face course, is to facilitate a better understanding of how microbeams work, what experiments can be performed using a microbeam, why these experiments are of biological interest, and how to design / perform these experiments.

The on-line material curriculum consists of audio podcasts and the same handouts that the face-to-face students received. The audio of each podcast is synched with the accompanying PowerPoint slides (viewable on a video iPod, tablet, PC or Mac, or smart phone), as well as a PDF version of the slides. High-resolution video (720p, with audio) was also used to document demonstrations of all aspects of a microbeam experiment, from making microbeam dishes to irradiating cells and performing online analyses. After extensive editing, this resulted in about 4½ hours of video footage. Additional material is added to the on-line course for new course presentations or lecturers.

The on-line training course can be accessed through the RARAF website (www.RARAF.org) and YouTube channel (http://www.youtube.com/user/RARAFcourses). The videos can be viewed on any Internet-enabled device supporting YouTube format.

Tours

In addition to training students, tours of the Facility provide a general introduction to the research performed at RARAF and the irradiation facilities that are available. This year we gave tours to more than 30 scientists, students, and members of the public.

As an example, high school seniors who had been offered priority admission to Columbia as physics majors, some of whom were Columbia I. I. Rabi Scholarship winners, toured RARAF in April along with Dr. John Parsons from the Physics Department at Nevis Labs.

Personnel

The Director of RARAF is Dr. David Brenner, the Director of the Center for Radiological Research (CRR). The accelerator facility is daily managed and operated by Dr. Gerhard Randers-Pehrson and Dr. Guy Garty, the Co-Associate Directors of RARAF.

Dr. Charles Geard, a Senior Biologist Emeritus, continues to visit RARAF frequently lending his considerable expertise.

Dr. Gerhard Randers-Pehrson, a Senior Research Scientist and Chief Physicist, is directly involved in the operations and new developments of RARAF. He lends his considerable expertise to all of the ongoing projects at RARAF.

Dr. Brian Ponnaiya, a Research Scientist, is the biology advisor for RARAF. He collaborates with many of the external users and coordinates with the CRR, where he spends about half his time.

Dr. Guy Garty, an Associate Professor, developed the Flow and Shoot (FAST) system and is developing the CE-LIF system. He spends about half his time working on the CRR Center for Medical Countermeasures against Radiation, for which he is the director of the Irradiation Core.

Dr. Andrew Harken, an Associate Research Scientist, is responsible for the Super Microbeam development with STED imaging. He is also the project leader on the microFACS system.

Dr. Manuela Buonanno, an Associate Research Scientist in radiation biology, collaborates with many external users and performs assays for the mouse ear microbeam irradiations.

Dr. David Welch, an Associate Research Scientist, is responsible for the development of new microfluidic tools and interfaces for microfluidic irradiation tools. His

expertise in microfluidics has been of considerable assistance in the development of our microfluidics applications.

Dr. Veljko Grilj, a Postdoctoral Research Scientist, is responsible for assisting Dr. Harken with the Super Microbeam development. He is also responsible for working with Drs. Ponnaiya and Buonanno operating the accelerator for outside user experiments.

Dr. Christian Siebenwirth, a Postdoctoral Research Scientist, is responsible for the DREEBIT Heavy Ion Source accelerator development project.

Dr. Malek Haj Tahar, a Postdoctoral Research Scientist, is responsible for assisting the modeling of RARAF ion beam systems. He will be taking the lead in the new development of a small animal irradiation therapy system as a potential future direction.

Ms. Sofia Barbieri, a Ph.D. Candidate at the University of Pavia in Italy, has joined us for a year and will be working on the microFACS project computer programming and user interface. She will also continue her Ph.D. work looking at H2AX focus formation with respect to particle LET.

Mr. Dennis Farrell has joined the RARAF staff on a part time basis. He is performing microbeam irradiations, serving as the Radiation Safety Officer and providing management support for the RARAF staff.

(l to r): Christian Siebenwirth, Andrew Harken, Guy Garty, and Veljko Grilj during an accelerator opening.

Two views of the open Singletron accelerator (above and right).